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In vitro efficacy of 2,*N*-bisarylated 2-ethoxyacetamides against *Plasmodium falciparum*

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ABSTRACT

Investigation of a series of 2,*N*-bisarylated 2-ethoxyacetamides resulted in the identification of four inhibitors **5**, **20**, **24**, **29** with single-digit micromolar in vitro efficacy against two drug-resistant *Plasmodium falciparum* strains. These compounds are analogs of structurally-related 1,3-bisaryl-2-propen-1-ones (chalcones), the latter showing efficacy in vitro but not in a malaria-infected mouse. The 2,*N*-bisarylated 2-ethoxyacetamides (e.g., **2**, **5**, **20**) were shown to possess significantly greater stability in the presence of metabolizing enzymes than the corresponding 1,3-bisaryl-2-propen-1-ones (e.g., **1**, **3**, **18**).

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Each year, more than 500,000 people die from malaria.[1] The organism responsible for most of these deaths, *Plasmodium falciparum*, has developed resistance to most available drugs.[2,3] Thus, there is an urgent need for novel and affordable new products.[4] Chalcones (1,3-diphenyl-2-propen-1-ones) and their carbocyclic and heterocyclic analogs display a wide range of biological activities, including antiprotozoal, antibacterial, antifungal and antiproliferative activity.[5–7] Numerous mechanisms of action for the chalcones have been proposed and supported with experimental data.[7–9] Several groups have studied the relationship between the structure of substituted chalcones and their antimalarial activity.[10–14] Chalcones possessing submicromolar efficacy against drug-resistant strains of *P. falciparum* in vitro have been identified. The emerging SAR does not correlate with the SAR of known antimalarial targets,[10,12] implying that the mechanism by which the chalcones act is distinct from established antimalarial mechanisms. Several of these compounds have been tested in animal models of malaria (*Plasmodium yoelii*- or *Plasmodium berghei*-infected mice),[10,12–14] and some possess significant in vivo efficacy.[12–14] However, in vitro and in vivo efficacies often do not correlate.[10,12] Although possibly due to the difference in *Plasmodium* species employed in in vitro and in vivo assays, we suspect that rapid compound metabolism also contributes, because the chalcones tested by us were all rapidly biotransformed when exposed to a preparation of human liver microsomes in vitro.[10] Despite the considerable interest in the biological activities of chalcones, few experimental studies of chalcone ADME, including metabolism, have been described.[15]

We therefore wished to identify a compound series structurally distinct from the chalcones, but that mediates antimalarial activity via the same mechanism. We aimed to achieve this through use of a 3D pharmacophore that we had developed using CATALYST software which models the structural and electronic features required by compounds to effect antimalarial activity via this novel chalcone-mechanism.[16]

Using this model we ascertained that the two aryl substituents appear to be involved in target binding, but not the linking 2-propen-1-one group. We therefore searched the Walter Reed Army Institute of Research's compound database for compounds possessing these substituents but containing an alternative linking group.[16,17] The 2,*N*-bisphenyl-2-ethoxyacetamides emerged as one possibility. In contrast to the rapid degradation of 1,3-bis-(4-chlorophenyl)-2-propen-1-one **1** upon exposure to a preparation of human liver microsomes in vitro, the corresponding 1,3-bis-(4-chlorophenyl)-2-ethoxyacetamide **2** exhibited significant stability. The potency of this amide **2** against *Plasmodium* in vitro was 2–3-fold lower than that of the chalcone **1**, but we had already learned which substitution patterns enhance the antiplasmodial potency of the chalcones.[10,16] We therefore proposed the synthesis

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of 2,*N*-bisaryl 2-ethoxyacetamides ('amides') containing these potency-enhancing substitution patterns, with the aim of producing potent, metabolically-resistant analogs of the potent, but metabolically-labile, 2-propen-1-ones ('chalcones').

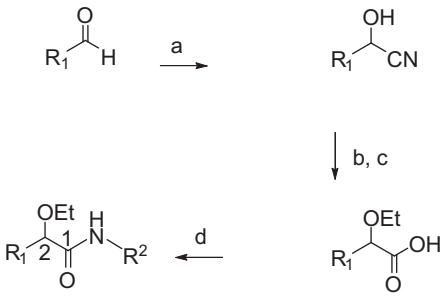
The most potent chalcones **3**, **8**, **18** previously identified contain either 2,5- or 3,4-dichlorophenyl-substitution proximal to the carbonyl of the chalcone linker (the R_1 position) with either 3- or 4-quinolinyl-substiution in the distal position (R_2).[10,16] Our modeling suggested that only the aryl substituents (and not the atoms of the linker) are involved in target binding,[16] but we nevertheless decided to produce both possible analogs of unsymmetrical amides, namely those possessing a dichlorophenyl-substituent at R_1 with a quinolinyl-substituent at R_2 and those with a dichlorophenyl-substituent at R_2 and a quinolinyl-substituent at R_1.

The preparation of the former series began with synthesis of the requisite dichloroglycolic acids, as described in the literature[18] and shown in Scheme 1.[19] Treatment of the compounds with diethyl sulfate, in the manner described with glycolic acid itself yielded the corresponding 2-ethoxyacetic acids.[18] Reaction with a variety of amines using standard peptide coupling reagents yielded the novel dichlorophenyl-*N*-quinolinyl-2-ethoxy-acetamides **5**–**7**, **10**–**12**, **20**–**22**, **25**–**27**, **30**–**32**.[20]

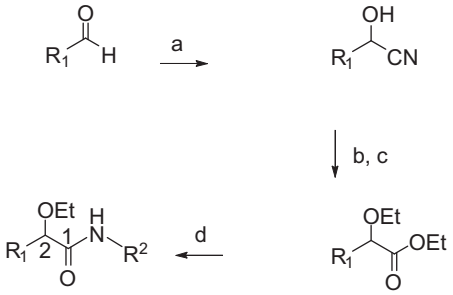
Preparation of the R_1/R_2-reversed compound series began by preparation of 4-quinolineglycolic acid as described in the literature[21] and shown in Scheme 2.[19] Ethylation of this compound using diethyl sulfate proved unsuccessful, but treatment with iodoethane in the presence of freshly prepared silver oxide yielded a bisethylated compound. This was directly reacted with various amines using a trimethyl aluminum catalyst to yield novel *N*-dichlorophenyl-4-quinolinyl-2-ethoxy-acetamides **9**, **19**, **29**.[22] We also discovered that the analogous reactions could be performed with the corresponding 3-quinoline-containing compounds. Thus, novel *N*-dichlorophenyl-3-quinolinyl-2-ethoxy-acetamides **4**, **14**, **24**, were also prepared.

The structures of the compounds prepared, and the results from their biological testing, are shown in Table 1. Compound identity, and assurance of purity exceeding 95%, was established by ^{1}H NMR and ^{13}C NMR. All the compounds were assayed in vitro against two strains of *P. falciparum*, and selected compounds for predicted metabolic stability by in vitro exposure to human and in some cases mouse liver microsome preparations.[23,24]

Most of the novel amides were active *in vitro* against *P. falciparum*, with several possessing single-digit micromolar (IC_{50} <10 - μM) in vitro efficacy against the two drug-resistant strains tested, the chloroquine, quinine and pyrimethamine-resistant W2 strain and the mefloquine-resistant D6 strain. (One determination of each IC_{50} was made; in other studies, when multiple determinations were made, standard deviations were generally small, such that a four-fold difference in activity is likely to be significant). As was previously seen with the chalcones, the efficacies of the amides



Scheme 1. Reagents and conditions: (a) aq $NaHSO_3$, ether; aq NaCN; (b) aq HCl; (c) Et_2SO_4, aq NaOH; (d) R_2NH_2, *N*-hydroxybenzotriazole, *o*-benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, *i*-Pr_2NEt.



Scheme 2. Reagents and conditions: (a) aq NH_4Cl, ether; aq NaCN, *i*-PrOH; (b) aq HCl; (c) EtI, Ag_2O; (d) R_2NH_2, Me_3Al, PhMe, CH_2Cl_2.

in the W2 and D6 strains were similar; a preliminary indication that the compounds are devoid of significant cross-resistance (Resistance indices = 1).[10]

The most potent chalcones **3**, **8** identified previously combine either 3- or 4-quinolinyl-substitution with 2,5-dichlorophenyl substitution. Activities of the 3-quinoline-containing amides **4**, **5** analogous to the 3-quinolinyl-chalcone **3** were compared, together with those of the analogous 2- and 6-quinoline-containing derivatives **6**, **7**. Similarly, activities of the 4-, 5- and 8-quinoline-containing amides (**9**–**10**, **11**, **12,** respectively) were compared to those of the analogous 4-quinolinyl chalcone **8**. All of the amide analogs (**4**–**7**, **9**–**12**) were found to be less potent than the chalcones (**3**,**8**) against both strains of drug-resistant parasite. The most active amide **5** was found to possess potency 10-fold lower against the D6 strain and six-fold lower against W2 than that of its corresponding chalcone **3**.

Additionally, it was found that reversing the location of R_1/R_2 has a minimal impact on activity; evidenced by the comparable activity of amide **4** versus **5**, and of amide **9** versus **10**, against both strains.

Other potent chalcones **13**, **18**, identified previously, combine either 3- or 4-quinolinyl-substitution with 3,4-dichlorophenyl substitution. Once again, the activities of these chalcones were compared with those of their analogous amides **14**–**17** and **19**–**22**, respectively. The range of potencies of these 3,4-dichlorophenyl-containing amides was greater than for their 2,5-dichlorophenyl-containing analogs **4**–**7**, **9**–**12**; with most of the amides being moderately active against both parasite strains. Notably, amide **20**, which possesses significant activity, is just two-fold less potent than its corresponding chalcone **13** against both strains. In contrast to the 2,5-dichlorophenyl-containing series, where the most potent amide **5** was *N*-substituted with a 3-quinolinyl-group; in the 3,4-dichlorophenyl series the most potent amide against both strains **20** was *N*-substituted with a 4-quinolinyl-group.

Finally the activities of the amides containing 2,4-dichloro-substitution **24**–**27**, **29**–**32** were compared with those of the analogous 3- and 4-quinolinyl-containing chalcones **23**, **28**. In these series the two most potent amides are more potent against both strains than their corresponding chalcones (**23** compared to **24**, **28** compared to **29**). In contrast to the previous series, these more active amides are *N*-substituted with the 2,4-dichlorophenyl-group and possess either 3- or 4-quinolinyl-substitution at the 2-position.

Having achieved the goal of producing amides with comparable in vitro antiplasmodial efficacy to the chalcones we have described previously,[10] the important question became how they compared in terms of predicted metabolic stability. Compound stability in the presence of human versus mouse liver microsomes was similar (**5**, **20**, **24**, **29**), so only the former was measured for most compounds. The same compounds, four of our most potent amides, were found to undergo biotransformation upon exposure to a